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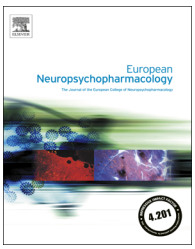

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Microglial activation in the rat brain following chronic antipsychotic treatment at clinically relevant doses

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Abstract
Neuroinflammation is increasingly implicated in the pathogenesis of Schizophrenia (SCZ). In addition, there is increasing evidence for a relationship between the dose and duration of antipsychotic drug (APD) treatment and reductions in grey matter volume. The potential contribution of microglia to these phenomena is however not yet defined. Adult rats were treated with a common vehicle, haloperidol (HAL, 2 mg/kg/day) or olanzapine (OLZ, 10 mg/kg/day) for 8 weeks via an osmotic mini-pump implanted subcutaneously. Microglial cells, identified by their Iba-1 immunoreactivity, were quantified in four regions of interest chosen based on previous neuroimaging data: the hippocampus, anterior cingulate cortex, corpus striatum, and secondary somatosensory cortex. Those cells were also analysed according to their morphology, providing an index of their activation state. Chronic APD treatment resulted in increased density of total microglia in the hippocampus, striatum, and somatosensory cortex, but not in the ACC. Importantly, in all brain regions studied, both APD tested led to a dramatic shift towards an amoeboid, reactive, microglial morphology after chronic treatment compared to vehicle-treated controls. These data provide the first in vivo evidence that chronic APD treatment at clinically relevant doses leads to microglial proliferation and morphological changes indicative of activated microglia in the naïve rat brain. Although caution needs to be exerted when extrapolating results from animals to patients, these data suggest a potential

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contribution of antipsychotic medication to markers of brain inflammation. Further investigation of the links between antipsychotic treatment and the immune system are warranted.

1. Introduction

Multiple lines of evidence suggest a role for the immune response and neuroinflammation in the pathogenesis schizophrenia (SCZ) (Brown and Derkits, 2010; Kahn and Sommer, 2014; Martins-de-Souza et al., 2009; Maxeiner et al., 2014; Miller et al., 2013a; Sommer et al., 2014). Microglia act as the resident macrophages of the brain and are the first cells to respond, mobilizing the inflammatory response to brain insult or injury (Venneti et al., 2006). This process is characterized by a switch in microglia phenotype from the quiescent or "resting" state to an activated, amoeboid state, including migration to the site of injury and the release of cytokines (Kettenmann et al., 2013). Microglial activation has been suggested to be involved in grey matter loss in a number of disorders, including SCZ (Kahn and Sommer, 2014; Lieberman et al., 2001; Wright et al., 2000). Cognitive symptoms in SCZ patients have also been linked to increases in markers of inflammation (Dickerson et al., 2007; Dickerson et al., 2012; Pedersen et al., 2008). Although early *post-mortem* studies reported an increased presence of microglia in the hippocampus and cortex of SCZ patients (Bayer et al., 1999; Busse et al., 2012; Radewicz et al., 2000), other studies have failed to replicate these differences (Arnold et al., 1998; Falke et al., 2000; Steiner et al., 2006; Wierzba-Bobrowicz et al., 2005) or even reported a decrease in microglial activation (Kurumaji et al., 1997).

Using positron emission tomography (PET), activated microglia may be indexed indirectly by the increased expression of 18 kDa translocator protein (TSPO) in microglial cells utilizing TSPO-specific radiotracers such as [^{11}C]-PK11195, ([^{11}C]-DAA1106) and [^{18}F]-FEPPA, that bind to it. (Papadopoulos et al., 2006). Studies using these approaches in SCZ patients are however mixed. Two studies using [^{11}C]-PK11195 report increased TSPO binding potential (BP), suggestive of activated microglial cells in patients with SCZ, particularly in the temporal lobe (Doorduin et al., 2009; van Berckel et al., 2008). Two subsequent studies utilizing second-generation TSPO radiotracers ([^{11}C]-DAA1106) (Takano et al., 2010) and [^{18}F]-FEPPA (Kenk et al., 2015), respectively, found no significant differences in the TSPO signal between SCZ patients and controls. These discrepancies may result from differences in patient populations, including the timing of the scan in relation to disease duration, methodological issues (in vivo vs. *post-mortem*) and additionally, the presence or absence of antipsychotic drugs (APD).

Importantly, there is now substantial evidence for a relationship between the dose and duration of APD treatment and reductions in cerebral grey matter volume as detected by magnetic resonance imaging (MRI), in patients with SCZ (Fusar-Poli et al., 2013; Ho et al., 2011). These data are supported by in vivo studies in rats using MRI (Vernon et al., 2011, 2012, 2014) and *post-mortem* data from primates (Dorph-Petersen et al., 2005), both using clinically comparable APD dosing regimens. The impact of chronic APD on brain microglia is however not yet well defined. While there is some evidence that APD may have some anti-inflammatory properties, these claims are often based on in vitro, non-clinical doses in vivo, or single doses of antipsychotics (Kato et al., 2011; Shin and Song, 2014; Venneti et al., 2006; Zhu et al., 2014) To our knowledge, no study has systematically examined the effect of current typical and atypical antipsychotics, at clinically relevant and chronic doses, on the density and morphology of microglia. In the current study, we therefore utilized *post-mortem* brain tissue from naïve rats treated chronically (8 weeks) with clinically comparable levels of haloperidol, olanzapine, or a common vehicle (Vernon et al., 2011) to undertake a rigorous *post-mortem* study of microglial density and morphology using design-based unbiased stereology. We examined multiple brain regions relevant to the pathogenesis of SCZ and previously shown to be impacted by chronic APD treatment in this rat model (Vernon et al., 2011, 2012, 2014). Our goal was to address two new questions: 1) Does chronic APD treatment lead to changes in microglia density and activation status? 2) Is this effect region specific?

2. Experimental procedures

2.1. Animals

Adult male Sprague-Dawley rats (Charles River UK, Kent, United Kingdom) initially weighing between 240 and 250 g were housed in groups of 4 animals per cage in a 12-h light-dark cycle. The housing facility was temperature- and humidity-controlled (21 ± 2 °C and 55 ± 10%, respectively). Animals were acclimatized to the unit for 7 days prior to experimentation. All procedures were undertaken in compliance with local ethical approval and with the Home Office Animals (Scientific Procedures) Act 1986, United Kingdom.

2.2. Chronic drug administration

Animals were randomly attributed to one of 3 treatment groups: vehicle (VEH), haloperidol (HAL), and olanzapine (OLZ); and dosed as previously described (Vernon et al., 2011). The data in this study represents findings from brain tissue harvested from these animals, as reported in our prior study of APD effects on brain volume using MRI (Vernon et al., 2011). No new animals were generated for the data presented herein.

2.3. Immunohistochemistry

A detailed description of the *post-mortem* tissue collection can be found elsewhere (Vernon et al., 2011). Briefly, a series of every twelfth section was used for the immunohistochemistry. Sections were washed in 0.1 M PBS and endogenous peroxidase activity was